Cyclosporin A Does Not Inhibit Epidermal Cell Growth at Therapeutic Levels

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The direct effect of cyclosporin A on epidermal cells was examined by using a pig skin explant culture system. A dose-response study using cyclosporin concentration from one-tenth the expected serum level to 100 times the expected serum level was done, and epidermal outgrowth and the number of mitotic figures were measured. Cyclosporin inhibited epidermal cell outgrowth at concentrations of 60 and 100 times the expected serum level, but it did not inhibit epidermal cell outgrowth at the serum level or even at 30 times the serum level. Cyclosporin did not inhibit mitotic figures significantly. These results suggest that cyclosporin does not inhibit epidermal cells directly.
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Recently, the widely used immunosuppressive agent cyclosporin A [1–3] has been reported to be effective in psoriasis [4–6] and ichthyosis [7] when it was used systemically. Although both psoriasis and ichthyosis are disorders of keratinization, there is a considerable difference between these 2 diseases; psoriasis is a disease thought to be related to T-lymphocyte function [8–11], but ichthyosis is thought to be only an epidermal disease. We present the results of the direct effects of cyclosporin on pig skin explant cultures. The lack of any direct effect will have to be explained in discussions of the beneficial effect of cyclosporin on these skin diseases.

MATERIALS AND METHODS

Skin slices were taken from the backs of small domestic pigs with a Castroviejo keratome set at a 0.2-mm depth. Multiple 2 × 2 mm skin pieces were prepared by cutting with a sharp scalpel and were placed dermis-side-down on glass coverslips in 3.5-cm Falcon Petri dishes and covered with 2 ml of culture medium. The growth medium consisted of RPMI 1640 with 10% fetal calf serum (FCS) plus antibiotics (penicillin 200 U/ml, streptomycin 200 μg/ml, amphotericin B 0.5 μg/ml, and mycostatin 100 U/ml). Cultures were incubated in a humidified incubator in an atmosphere of 95% air/5% carbon dioxide. After 96 h of growth, we measured the outgrowth of each explant and the medium was changed to new medium with 5% FCS plus antibiotics and containing different concentrations of cyclosporin. After an additional 72 h of growth, each outgrowth was measured again, and 1–3 μg/ml colchicine was added for 4 h. After fixation and staining, mitotic figures were counted using essentially the same methods described in [12,13].

Fetal calf serum, RPMI 1640 media, and antibiotics were obtained from GIBCO (Grand Island, New York), colchicine was obtained from Eli Lilly and Company (Indianapolis, Indiana), and cyclosporin (intravenous agent) was obtained from Sandoz Inc. (East Hanover, New Jersey). All other chemicals were of analytical reagent grade.

RESULTS

We carried out a dose-response study using concentrations of cyclosporin from one-tenth the expected serum level to 100 times the serum level. Table I shows the results of 7 different experiments. Serum level was taken as 200 ng/ml according to previous reports [14,15]. There was no inhibitory effect on the outgrowth of explants up to a concentration of 30 times the serum level compared with the control, but there were inhibitory effects at concentrations of 60 and 100 times the serum level. The mean percentage of outgrowth at 60 times is 43 ± 6.98(SEM)%, and the mean at 100 times is −12 ± 8.37(SEM)% compared with control.

Figure 1 shows the typical explant samples of the normal control (*A*), at the serum level of cyclosporin (*B*), and at 100 times the serum level of cyclosporin (*C*) after 72 h of incubation. The length of outgrowth is less at 100 times the serum level, and the number of layers from the edges of the original skin was greater than normal control or serum level. When we changed the medium from 100 times the serum level to a new control medium again after 72 h of incubation, explants started to grow again and the thickness of the layers became normal. This suggests that the explants were not killed by 100 times the serum level of cyclosporin; they were only inhibited from growing during direct exposure to the drug.

After measuring the outgrowth, the medium was changed to a medium containing 1–3 μg/ml colchicine and the tissue was incubated for 4 h at 37°C. After staining with 1% toluidine blue the mitotic figures were counted (Table II). In 2 of 3 experiments with 3 μg/ml of colchicine there was no inhibition of mitosis (Ex. 6 and Ex. 7). In the single experiment with 1 μg/ml colchicine (Ex. 4), both 60 and 100 times the serum concentration showed slight inhibition. Mitotic figures after colchicine incubation were more easily seen with 3 μg/ml of colchicine. There is, therefore, either no effect at all on mitotic activity or a slight effect at high concentrations that is less than the effect on the outgrowth size.

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Abbreviation:

FCS: fetal calf serum

Table I. Effect of Cyclosporin on Epidermal Outgrowth

	Ex. 1	Ex. 2	Ex. 3	Ex. 4	Ex. 5	Ex. 6	Ex. 7
Control	100 (9)	100 (13)	100 (12)	100 (14)	100 (14)	100 (8)	100 (17)
$\times\frac{1}{10}$	124 (7)		114 (13)	129 (10)			153 (14)
Serum 1	107 (8)	96 (17)	153 (10)	115 (14)		87 (9)	100 (15)
×10	103 (9)	88 (13)	143 (14)	121 (17)	115 (7)	92 (13)	143 (13)
×30		101 (16)		97 (18)	105 (7)	102 (9)	107 (16)
×60		59 (12)		44 (15)	23 (7)	57 (10)	32 (16)
×100	−41 (8)	4 (12)	−1 (12)	−27 (16)		−21 (8)	12 (17)

After a 72-h incubation in each medium with or without cyclosporin, the length of the explant outgrowth was measured and expressed as a percentage of each control value. The number of samples is in parentheses. The microns of outgrowth of each control were as follows: Ex. 1 = 442.2, Ex. 2 = 381.6, Ex. 3 = 301.6, Ex. 4 = 222.0, Ex. 5 = 476.0, Ex. 6 = 292.0, Ex. 7 = 246.0. Serum level of cyclosporin was taken as 200 ng/ml.

DISCUSSION

Cyclosporin A is an immunosuppressive agent widely used in human organ transplantation [1–3]. The mechanism of action of this drug is now under investigation by many groups. Merker and Handschumacher [16] observed, by using the murine thymoma cell line, that most of the drug concentrated by cells is located in the cytosol, and Krönke et al [17] reported that it inhibits T-cell growth factor gene expression at the level of mRNA transcription. Also cyclosporin has been used for treatment of autoimmune diseases [2,18]. In dermatology, oral use of cyclosporin for graft-versus-host disease [19] and the treatment of cutaneous T-cell lymphoma [20] were reported.

Moreover, cyclosporin was reported recently to be very effective in treating psoriasis when it was used systemically [4–6], and it was also reported to cause improvement in ichthyosis [7]. Since psoriasis and ichthyosis are both diseases with disorders of keratinization, we examined the effect of cyclosporin directly on the epidermal cells.

Although cyclosporin inhibited epidermal cell outgrowth at concentrations 60 and 100 times the serum level (Table I) or mitoses (Table II) in our study, it could not inhibit cell outgrowth at the serum level or even at 30 times the serum level. Cyclosporin is therefore unlikely to act in psoriasis and in ichthyosis by a direct effect on epidermal cells.

Although the primary defect in psoriasis is usually thought to be enhanced epidermal cell proliferation, psoriasis has also been reported to have a defective function of T lymphocytes [8] and an increased helper–suppressor T-cell ratio [10]. Discussions of a possible immune basis were reported [9,11]. So it may be that we can explain the effect of cyclosporin on psoriasis through inhibition of T-lymphocyte action.

Table II. Effect of Cyclosporin on Mitotic Figures

	Ex. 4	Ex. 6	Ex. 7
Control	4.1 (14)	3.0 (8)	2.8 (17)
$\times\frac{1}{10}$	3.2 (10)		3.4 (14)
Serum 1	3.0 (14)	2.6 (9)	3.1 (15)
×10	2.9 (17)	2.9 (13)	3.2 (13)
×30	2.5 (18)	3.2 (9)	3.4 (16)
×60	1.6 (15)	3.2 (10)	4.2 (16)
×100	1.7 (16)	2.8 (8)	3.4 (17)

After a 72-h incubation in each medium with or without cyclosporin, the medium was changed to medium containing 1 (Ex. 4) or 3 (Ex. 6 and Ex. 7) μg/ml colchicine and the tissue was incubated for 4 h at 37°C. After staining with 1% toluidine blue the numbers of mitotic figures were counted. The number of samples is in parentheses.

Though severe toxicities of cyclosporin have been reported [15,21–24], more topical dermatologic trials may be worthwhile, as has been pointed out by Aldridge et al [25]. However, the amount of the drug reaching the systemic circulation by topical application may become equal to oral administration.

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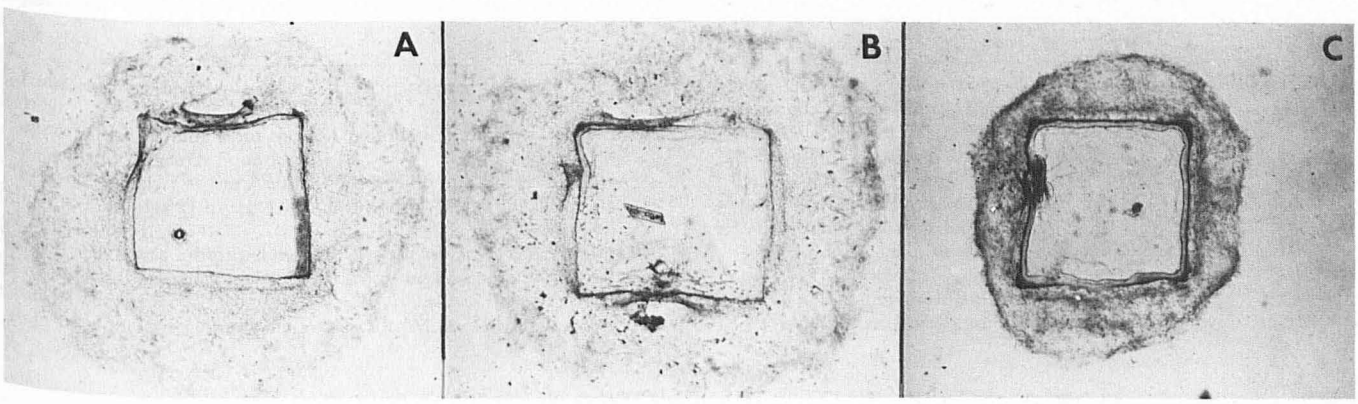

Figure 1. Explants from 3 groups. The explants were grown for 96 h in RPMI 1640 with 10% FCS plus antibiotics (penicillin 200 U/ml, streptomycin 200 μg/ml, amphotericin B 0.5 μg/ml, and mycostatin 100 U/ml) and the length from the edge of the original skin explant to the top of the newly outgrown layers was measured. Then each medium was changed to new medium with or without cyclosporin. After 72 h of incubation, the length of the outgrowth was measured again, and explants were stained with 1% toluidine blue. *A*, Control culture; *B*, culture with serum level of cyclosporin; *C*, culture with 100 times the serum level of cyclosporin.

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